AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

These claims are copied from US Patent Nos. 6,544,785 and 6,649,372 (with minor modifications, e.g., to change claim numbers). Support for various aspects of the copied claims is replete throughout the subject specification, claims, and drawings as originally filed. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with any objection or rejection of record.

Listing of Claims:

1-34. (Cancelled).

35. (New) A method for rescuing a recombinant negative strand RNA virus comprising:

(a) introducing into a 293 cell expression vectors which direct the expression in said cells of genomic or antigenomic vRNA segments, and a nuclcoprotein, and an RNA-dependent polymerase, so that ribonucleoprotein complexes can be formed and viral particles can be assembled in the absence of helper virus; and

(b) culturing said cells wherein viral particles are packaged and rescued.

36. (New) The method of claim 35 wherein the recombinant negative strand RNA virus is a segmented virus.

37. (New) The method of claim 36 wherein the negative strand RNA virus is influenza.

38. (New) A method for generating in cultured cells infectious viral particles of a segmented negative-strand RNA virus having greater than 3 genomic vRNA segments, said method comprising:

(a) introducing into cultured cells expression vectors which direct the expression of the genomic or antigenomic vRNA segments of said virus, and a nucleoprotein, and an RNA dependent polymerase so that RNP complexes containing the genomic vRNA segments of said virus can be formed and said viral particles, can be assembled within said cells in the absence of helper virus; and

(b) culturing said cells wherein said viral particles are produced.

39. (New) The method of claim 38 wherein one or more further expression vectors are employed in said cells to express one or more proteins selected from said nucleoprotein and the subunits of said RNA-dependent RNA polymerase.

40. (New) The method of claim 38 wherein a cell line is employed which contains expression vectors which direct expression of one or more of said nucleoprotein and the subunits of said RNA-dependent RNA polymerase.

41. (New) The method of claims 38, 39 or 40 wherein said virus is an influenza virus of type A, B or C.

42. (New) The method of claim 38 wherein said virus is a reassortant virus having vRNA segments derived from more than one parent virus

43. (New) The method of claim 38 wherein said expression vectors direct expression of genomic vRNA segments of said virus.

44. (New) The method of claim 38 which further comprises amplifying viral particles produced by said cells by one or more further cellular infection steps employing cells which are the same or different from said first population of cells.

45. (New) The method of claim 38 which further comprises isolating infectious viral particles.

46. (New) The method of claim 38 which further comprises a viral attenuation or killing step.

47. (New) The method of claim 38 wherein said expression vectors are all plasmids.

48. (New) The method of claim 38 wherein said expression vectors consist of a separate expression vector for expression of each vRNA segment of said virus or the corresponding cRNAs.

49. (New) A method for rescuing a chimeric recombinant negative strand RNA virus, wherein said chimeric virus expresses heterologous nucleic acid sequences, comprising:

(a) introducing into a 293 cell, expression vectors which direct the expression in said cells of genomic or antigenomic vRNA segments, and a nucleoprotein, and an RNA-dependent RNA polymerase, so that ribonucleoprotein complexes can be formed and viral particles can be assembled in the absence of helper virus; and

(b) culturing said cells wherein viral particles are packaged and rescued.

50. (New) The method of claim 49, wherein said heterologous nucleic acid sequences are inserted into a DNA complement of a negative strand RNA virus gene, such that said heterologous nucleic acid sequences are flanked by the viral polymerase binding site, and a polyadenylation site.

51. (New) The method of claim 49, wherein oligonucleotides encoding the viral polymerase binding site of the negative strand RNA virus are ligated to said heterologous nucleic acid sequences.

52. (New) The method of claim 49, wherein the chimeric recombinant negative strand RNA virus is a segmented virus.

53. (New) The method of claim 52, wherein the segmented RNA virus is influenza.

54. (New) The method of claim 49, wherein said heterologous nucleic acid sequences are derived from human immunodeficiency virus (HIV).

55. (New) The method of claim 49, wherein said pathogenic antigens are hepatitis B surface antigen, glycoproteins of herpes virus, or VP1 protein of poliovirus.

56. (New) The method of claim 49, wherein said non-viral pathogens are bacteria or parasites.

57. (New) The method of claim 49, wherein said heterologous nucleic acid sequences encode viral genes from different strains of the negative strand RNA virus.

58. (New) The method of claim 49, wherein said heterologous nucleic acid sequences are antisense nucleic acids.

59. (New) The chimeric recombinant negative strand RNA virus produced by the method of claim 49.

60. (New) A method for generating in cultured cells infectious viral particles of a chimeric negative strand RNA virus, wherein said chimeric virus expresses heterologous nucleic acid sequences and has greater than 3 genomic vRNA segments, comprising:

(a) introducing into cultured cells expression vectors which direct the expression of genomic or antigenomic vRNA segments of said chimeric virus, and a nucleoprotein, and an RNA dependent RNA polymerase so that RNP complexes containing the genomic vRNA segments of said chimeric virus can be formed and said viral particles can be assembled within said cells in the absence of helper virus; and

(b) culturing said cells wherein said viral particles are produced.

61. (New) The method of claim 60, wherein one or more further expression vectors are employed in said cells to express one or more proteins selected from said nucleoprotein and the subunits of said RNA-dependent polymerase.

62. (New) The method of claim 60, wherein a cell line is employed which contains expression vectors which direct expression of one or more of said nucleoprotein and the subunits of said RNA-dependent RNA polymerase.

63. (New) The method of claim 60, 61, or 62 wherein said virus is an influenza virus of type A, B or C.

64. (New) The method of claim 60, wherein said expression vectors direct expression of genomic vRNA segments of said chimeric virus.

65. (New) The method of claim 60 which further comprises amplifying viral particles produced by said cells by one or more further cellular infection steps employing cells which are the same or different from said first population of cells.

66. (New) The method of claim 60, which further comprises isolating infectious viral particles.

67. (New) The method of claim 60, which further comprises a viral attenuation or killing step.

68. (New) The method of claim 60, wherein said expression vectors are all plasmids.

69. (New) The method of claim 60, wherein said expression vectors consist of a separate expression vector for expression of each vRNA segment of said chimeric virus or the corresponding cRNAs.

70. (New) The method of claim 60, wherein said heterologous nucleic acid sequences are engineered into the expression vectors directing the expression of the vRNA segments of said chimeric virus.

71. (New) The method of claim 53 which further comprises amplifying viral particles produced by said first population of cells by one or more further cellular infection steps employing cells which are the same or different from said first population of cells.

72. (New) The method of claim 53 which further comprises isolating infectious viral particles.

73. (New) The method of claim 53 which further comprises an attenuation or viral killing step.

74. (New) The method of claim 53 which further comprises incorporating attenuated or killed vial particles into a vaccine composition.

75. (New) A vaccine formulation comprising the chimeric recombinant negative strand RNA virus produced by the method of claim 49.

76. (New) A vaccine formulation comprising the chimeric recombinant negative strand RNA virus produced by the method of claim 49, wherein said heterologous nucleic acid sequences are derived from human immunodeficiency virus.

77. (New) A vaccine formulation comprising the chimeric recombinant negative strand RNA virus produced by the method of claim 49, wherein said pathogenic antigen is hepatitis B surface antigen, glycoprotein of herpes virus, or VP1 protein of poliovirus.

78. (New) The vaccine formulation comprising the chimeric recombinant negative strand RNA virus produced by the method of claim 49, wherein said virus is influenza.

79. (New) A pharmaceutical composition comprising, the chimeric recombinant negative strand RNA virus produced by the method of claim 49, and a pharmaceutically acceptable carrier.